The Arabidopsis leaf transcriptome reveals distinct but also overlapping responses to colonization by phyllosphere commensals and pathogen infection with impact on plant health

Vogel, Christine ; Bodenhausen, Natacha ; Gruissem, Wilhelm ; Vorholt, Julia A

Abstract: Plants are colonized by a variety of bacteria, most of which are not pathogenic. Currently, the plant responses to phyllosphere commensals or to pathogen infection in the presence of commensals are not well understood. Here, we examined the transcriptional response of Arabidopsis thaliana leaves to colonization by common commensal bacteria in a gnotobiotic system using RNA sequencing and conducted plant mutant assays. Arabidopsis responded differently to the model bacteria Sphingomonas melonis Fr1 (S.Fr1) and Methylobacterium extorquens PA1 (M.PA1). Whereas M.PA1 only marginally affected the expression of plant genes (< 10), S.Fr1 colonization changed the expression of almost 400 genes. For the latter, genes related to defense responses were activated and partly overlapped with those elicited by the pathogen Pseudomonas syringae DC3000 (Pst). As S.Fr1 is able to mediate plant protective activity against Pst, we tested plant immunity mutants and found that the pattern-recognition co-receptor mutant bak1/bkk1 showed attenuated S.Fr1-dependent plant protection. The experiments demonstrate that the plant responds differently to members of its natural phyllosphere microbiota. A subset of commensals trigger expression of defense-related genes and thereby may contribute to plant health upon pathogen encounter.

DOI: https://doi.org/10.1111/nph.14036

New Phytologist

The Arabidopsis leaf transcriptome reveals distinct but also overlapping responses to colonization by phyllosphere commensals and pathogen infection with impact on plant health

Christine Vogel[1], Natacha Bodenhausen[1], Wilhelm Gruissem[2] and Julia A. Vorholt[1]

[1]Department of Biology, Institute of Microbiology, ETH Zurich, 8093 Zurich, Switzerland; [2]Department of Biology, Institute of Agricultural Sciences, ETH Zurich, 8092 Zurich, Switzerland

Author for correspondence:
Julia A. Vorholt
Tel: +41 (0)44 632 55 24
Email: jvorholt@ethz.ch

Received: *14 January 2016*
Accepted: *25 April 2016*

New Phytologist (2016) **212:** 192–207
doi: 10.1111/nph.14036

Key words: Arabidopsis, commensal bacteria, microbe-associated molecular pattern (MAMP), plant microbiota, plant pathogen, plant protection, transcriptional profiling.

Summary

- Plants are colonized by a variety of bacteria, most of which are not pathogenic. Currently, the plant responses to phyllosphere commensals or to pathogen infection in the presence of commensals are not well understood.
- Here, we examined the transcriptional response of *Arabidopsis thaliana* leaves to colonization by common commensal bacteria in a gnotobiotic system using RNA sequencing and conducted plant mutant assays.
- Arabidopsis responded differently to the model bacteria *Sphingomonas melonis* Fr1 (S.Fr1) and *Methylobacterium extorquens* PA1 (M.PA1). Whereas M.PA1 only marginally affected the expression of plant genes (< 10), S.Fr1 colonization changed the expression of almost 400 genes. For the latter, genes related to defense responses were activated and partly overlapped with those elicited by the pathogen *Pseudomonas syringae* DC3000 (Pst). As S.Fr1 is able to mediate plant protective activity against Pst, we tested plant immunity mutants and found that the pattern-recognition co-receptor mutant *bak1/bkk1* showed attenuated S.Fr1-dependent plant protection.
- The experiments demonstrate that the plant responds differently to members of its natural phyllosphere microbiota. A subset of commensals trigger expression of defense-related genes and thereby may contribute to plant health upon pathogen encounter.

Introduction

In nature, plants are colonized by a variety of organisms such as bacteria and fungi. Bacteria represent the highest fraction of plant colonizers in the phyllosphere, the aboveground parts of plants (Lindow & Brandl, 2003; Vorholt, 2012; Bulgarelli *et al.*, 2013; Leveau, 2015). These plant-associated organisms might influence plants in various ways and it is increasingly clear that the phyllosphere microbiota has beneficial effects for plants including growth promotion or protection against biotic and abiotic stress (Innerebner *et al.*, 2011; Penuelas & Terradas, 2014; Schlaeppi & Bulgarelli, 2015; Ritpitakphong *et al.*, 2016).

Studies on plant responses to bacterial colonization historically have focused mainly on phytopathogens, looking at the short-term response to treatment with virulent, avirulent or nonhost pathogens (e.g. Tao *et al.*, 2003; Thilmony *et al.*, 2006; Truman *et al.*, 2006). Upon pathogen encounter, plants elicit an immune response to limit pathogen growth. Whereas biotrophic and hemibiotrophic pathogens are combatted mainly by salicylic acid (SA)-dependent defense responses, necrotrophic pathogens are generally sensitive to jasmonic acid (JA) and ethylene (ET)-dependent defense responses (Glazebrook, 2005; Jones & Dangl, 2006; Pieterse *et al.*, 2012). Pathogens are first perceived by pattern-recognition receptors (PRRs) that bind pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs), initiating a layer of basal defense called pattern-triggered immunity (PTI). To cause disease, pathogens inject effectors into plant cells that interfere with PRR complexes or downstream signaling to overcome PTI. Plants have in turn evolved resistance genes whose products recognize these effectors either directly or indirectly, resulting in effector-triggered immunity (ETI), a more specific defense response often accompanied by a hypersensitive response (HR) (Jones & Dangl, 2006; Boller & Felix, 2009; Dodds & Rathjen, 2010; Böhm *et al.*, 2014).

Most bacteria colonizing plants in the wild are not pathogenic (e.g. Vorholt, 2012). Many of these are predicted to produce MAMPs, raising the question of how plants distinguish between pathogens and nonpathogens in the first place. In several studies, responses of Arabidopsis to colonization by different root-colonizing bacteria able to induce a systemic resistance effective against *Pseudomonas syringae* and other pathogens were investigated (Cartieaux *et al.*, 2003, 2008; Verhagen *et al.*, 2004; Wang *et al.*, 2005b; van de Mortel *et al.*, 2012; Weston *et al.*, 2012). In most of these, only few transcriptional changes were observed in systemic leaf tissues after root colonization. Verhagen *et al.* (2004), for example, did not find any differentially expressed

genes in the leaves upon root colonization by *P. fluorescens* WCS417r, but observed gene expression changes in the roots, with most genes downregulated. Cartieaux *et al.* (2003) found differential expression for 63 genes in shoots and only few gene expression changes in the roots of *P. thivervalensis*-colonized plants. Both of these strains induce a systemic resistance response that is dependent on JA- and ET-signaling. By contrast, many more regulated genes were found in another study (van de Mortel *et al.*, 2012), in which the transcriptional long-term response to colonization by *P. fluorescens* SS101 was examined. This strain induces systemic resistance by a different, SA-signaling dependent mechanism.

Here, we analyzed the transcriptional response of *Arabidopsis thaliana* to colonization by indigenous phyllosphere isolates. The two genera *Methylobacterium* and *Sphingomonas* can be found abundantly in the phyllosphere of plant species including Arabidopsis (Delmotte *et al.*, 2009). Members of both genera have been identified as phyllosphere community members in several other plant species and are thought to constitute part of the core phyllosphere community (Kim *et al.*, 1998; Knief *et al.*, 2010a,b; Vorholt, 2012; Bodenhausen *et al.*, 2013; Bulgarelli *et al.*, 2013). The two genera have adapted differently to the plant environment. While *Methylobacterium* are benefiting from a methylotrophic lifestyle with the utilization of methanol released by pectin metabolism, *Sphingomonas* produce a vast array of transporters such as TonB-dependent transporters that are associated with the scavenging of the few carbon sources available in the phyllosphere (Delmotte *et al.*, 2009). Several members of the genus *Sphingomonas* originally isolated from plants conferred plant protection against *P. syringae* DC3000 and *Xanthomonas campestris* in a gnotobiotic model system, whereas no plant protection was observed by colonization with members of the genus *Methylobacterium* (Innerebner *et al.*, 2011). We therefore chose to use two well-studied model strains as representatives, *Methylobacterium extorquens* PA1 and *Sphingomonas melonis* Fr1 that both have been isolated from Arabidopsis (Knief *et al.*, 2010a; Innerebner *et al.*, 2011). We describe here the transcriptional response of Arabidopsis leaves to colonization by these model strains under gnotobiotic conditions and compare it to the response elicited by the plant pathogen *P. syringae* DC3000. In addition, we included plants colonized by commensals and challenged by *P. syringae* to determine whether the presence of commensals influences the transcriptional response to *P. syringae*.

Materials and Methods

Plant material and growth conditions

Plants (*Arabidopsis thaliana* Col-0) were cultivated in full-gas microboxes (Combiness) as described (Innerebner *et al.*, 2011). The mutants *eds1-2/pad4-1* and *jar1-1* were provided by Philippe Reymond (University of Lausanne, Switzerland), *bak1-5/bkk1-1* and *fls2c/efr-1/cerk1-2* were obtained from Cyril Zipfel (The Sainsbury Laboratory, Norwich, UK), *sid2-1* from Christiane Nawrath (University of Lausanne, Switzerland) and *sobir1-12*, *rlp23-1* and *rlp23-2* seeds were provided by Thorsten Nürnberger (University of Tübingen, Germany). With the exception of *rlp23-1* and *rlp23-2*, all plant mutants were tested at least twice with similar results. Results of one representative experiment are shown. After inoculation of seeds (see later), the boxes were transferred to a climate chamber with the temperature set to 22°C. After 1 wk of cultivation, the photoperiod was changed from long day (16 h) to short day (9 h photoperiod). One day before infection with *Pseudomonas syringae* DC3000 lux (Pst, Fan *et al.*, 2008) or mock-treatment, 30 holes were punched into microbox walls with a canula (diameter 1.2 mm) to reduce relative humidity within the box.

Plant inoculation

Plants were seed-inoculated with suspensions of the commensals *Sphingomonas melonis* Fr1 (S.Fr1) or *Methylobacterium extorquens* PA1 (M.PA1) as described (Innerebner *et al.*, 2011). After 3 wk, plants were spray-infected with Pst or mock-treated with 10 mM $MgCl_2$. A lawn of Pst was grown on King's B (King *et al.*, 1954) plate overnight at 28°C. The infection suspension was prepared from cells collected in 10 mM $MgCl_2$ and the concentration was adjusted to an optical density at 600 nm of 0.001. Plants were infected by spraying four times with a chromatographic TLC reagent sprayer under sterile conditions just before the start of the light period.

Bacterial growth determination

Bacterial colonization on the aerial plant parts was determined as described previously (Innerebner *et al.*, 2011). For axenic plants, 50–100 µl of the solutions were additionally plated on nutrient broth plates and incubated at 28°C for 1 wk to check for contaminations. Only leaves from confirmed axenic plants were used for RNA extraction of this treatment.

Plant sampling for RNASeq

Four independent experiments were conducted to collect plant material for transcriptome analysis, with each corresponding to one biological replicate. The samples consisted of leaves from seed-inoculated plants (with S.Fr1, M.PA1 or 10 mM $MgCl_2$ (axenic control), treated with Pst and a spray of 10 mM $MgCl_2$ (CTL), respectively). Leaves for RNA extraction were collected at 2 and 7 d post spray-infection (T1 and T2) at midday. For each treatment, single leaves were removed from 18 plants (out of three microboxes), directly frozen in liquid nitrogen and then stored at −80°C. Total RNA was extracted from 18 pooled leaves using the RNeasy mini Plant kit (Qiagen) including the on-column DNaseI digest according to the manufacturer's recommendations.

Illumina sequencing

RNA quality assessment and sequencing using an Illumina HiSeq2000 is described in Supporting Information Methods S1. Counts of mapped reads are given in Table S1.

Differential expression analysis

Genes containing at least 0.5 counts per million in at least four samples (in total 19 476 genes) were considered for differential expression analysis using the Bioconductor software package EDGER (v.3.6.4) (Robinson *et al.*, 2010) within the R environment (v.3.1.0; R Development Core Team, http://www.R-project.org). The normalization factors were calculated by trimmed mean of M values (TMM) method. The gene-wise dispersions were estimated by Cox–Reid approximate conditional maximum-likelihood and an empirical Bayes procedure was used to shrink the dispersions towards trended values based on expression level and the differential expressions were assessed at both time points using likelihood ratio tests on a fitted generalized linear model with accounted batch effect, using the appropriate contrasts (McCarthy *et al.*, 2012).

Genes showing an absolute fold change (FC) > 2 with an adjusted (Benjamini and Hochberg method) *P*-value (false discovery rate, FDR) < 0.05 were considered as differentially expressed. Genes with an FDR < 0.05 at both time points and showing absolute FC > 2 at one time point and FC > 1.5 at the other time point were considered as consistently regulated by a given treatment.

Moderated $\log_2$-transformed counts per million were used for cluster analysis and heatmaps. To avoid taking the log of 0 counts, a prior count of 2 was added first. Euclidian distances were calculated and clustered with the function hclust in the R environment using the method 'WARD.D2'. For heatmaps, samples were clustered based on Pearson correlation using the function HEATMAP.2. Multidimensional scaling (MDS) plots were made using the EDGER package.

Genes identified as differentially expressed were subjected to GO over-representation analysis within the plugin BiNGO (v.2.44) within CYTOSCAPE (v.2.8.3) (Maere *et al.*, 2005; Smoot *et al.*, 2011). GO associations with evidence code inferred from electronic annotation were discarded before analysis using a hypergeometric test and Benjamini–Hochberg false discovery correction (FDR < 0.05). Lists of regulated genes were tested for over-representation against the whole GO annotation of biological processes (Table S2). In addition, all GO-terms with FDR < 0.05 were used as input for REVIGO (Supek *et al.*, 2011), which summarizes the GO-terms by clustering based on semantic similarities to reduce the list to the most representative GO-terms (Tables S3, S4).

Validation of sequencing data

Quantitative reverse transcription polymerase chain reaction (Table S5) performed in dynamic arrays were used to confirm the findings of RNA sequencing on a subset of genes as described in Methods S2 with primers shown in Table S6.

Accession number

RNA sequencing data are available in the ArrayExpress database (http://www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-4683.

Results

M.PA1 and S.Fr1 colonization induce Arabidopsis gene expression changes to different extent

We first analyzed to what degree the plants responded to colonization by the commensals M.PA1 and S.Fr1 in comparison to axenically grown plants using RNA-Seq technology. In addition, the changes triggered by the commensals were contrasted with the response provoked by the pathogen Pst. We collected leaf material from four independent experiments for transcriptome analysis. Plants were grown in a gnotobiotic model system, in which the commensals were seed-inoculated ensuring uniform leaf colonization. Samples for transcriptome analysis were taken at 23 and 28 d post sowing (T1 and T2; Fig. 1a). Both commensals colonized Arabidopsis leaves in the range of 10^8 colony forming units (CFU) per gram fresh weight (Fig. 1c). We chose a low Pst inoculation titer corresponding to a *c.* 100 times lower colonization compared to S.Fr1 and M.PA1 at T1 (corresponding to 2 d post spray-infection with Pst) to mimic a sporadic pathogen infection, which, however, increased to 10^9 CFU g^{-1} at T2 (7 d post spray-infection with Pst) in axenic plants (Fig. 1c,e). The axenic plants infected with the pathogen showed phenotypic differences, whereas commensal-colonized plants looked healthy (Figs 1b,d, S1), as expected (Innerebner *et al.*, 2011).

Hierarchical clustering of the Arabidopsis leaf transcriptomes separated the treatments into three main clusters: one containing axenic and M.PA1-colonized plants, one comprising S.Fr1-colonized plants and one comprising plants infected with Pst (Fig. 2a), indicating that the plant response to these organisms differed. An MDS plot separated in the first dimension mainly Pst-infected plants and in the second S.Fr1-inoculated plants from all other treatments (Fig. 2b). High reproducibility of the transcriptome of axenic and M.PA1-inoculated plants, as well as Pst-infected plants was evident at T1, whereas for S.Fr1-inoculated plants and Pst-infected plants at T2 the variation was higher. The analyses also indicated that differentially expressed genes (DEGs) compared to axenic controls can be found for S.Fr1-inoculated and Pst-infected plants rather than for M.PA1-inoculated plants. Colonization with M.PA1, S.Fr1 and Pst resulted in 37, 617 and 416 DEGs at T1 and in 6, 421 and 1809 at T2, respectively (Tables 1, S7; FDR < 0.05 and absolute FC > 2). The majority of regulated genes were induced in response to bacterial colonization. For Pst, 99% and 71% of genes were induced at T1 and T2, whereas 89% of genes were induced by the commensal S.Fr1.

At T1, most DEGs were specific to one treatment (Figs 3a,c, S2a,c), revealing distinct responses with little overlap. However, as pointed out above, Pst cell numbers increased between the first and second time point approaching those of the commensals by 7 d post spray-infection (Fig. 1c,e). Notably, at T2, the number of S.Fr1 DEGs shared with the DEGs in response to Pst increased to 245, with 12 showing opposite directions of regulation (Fig. S2b). Four of six DEGs in response to M.PA1 at T2 were also differentially expressed in at least one of the other treatments (Fig. S2b).

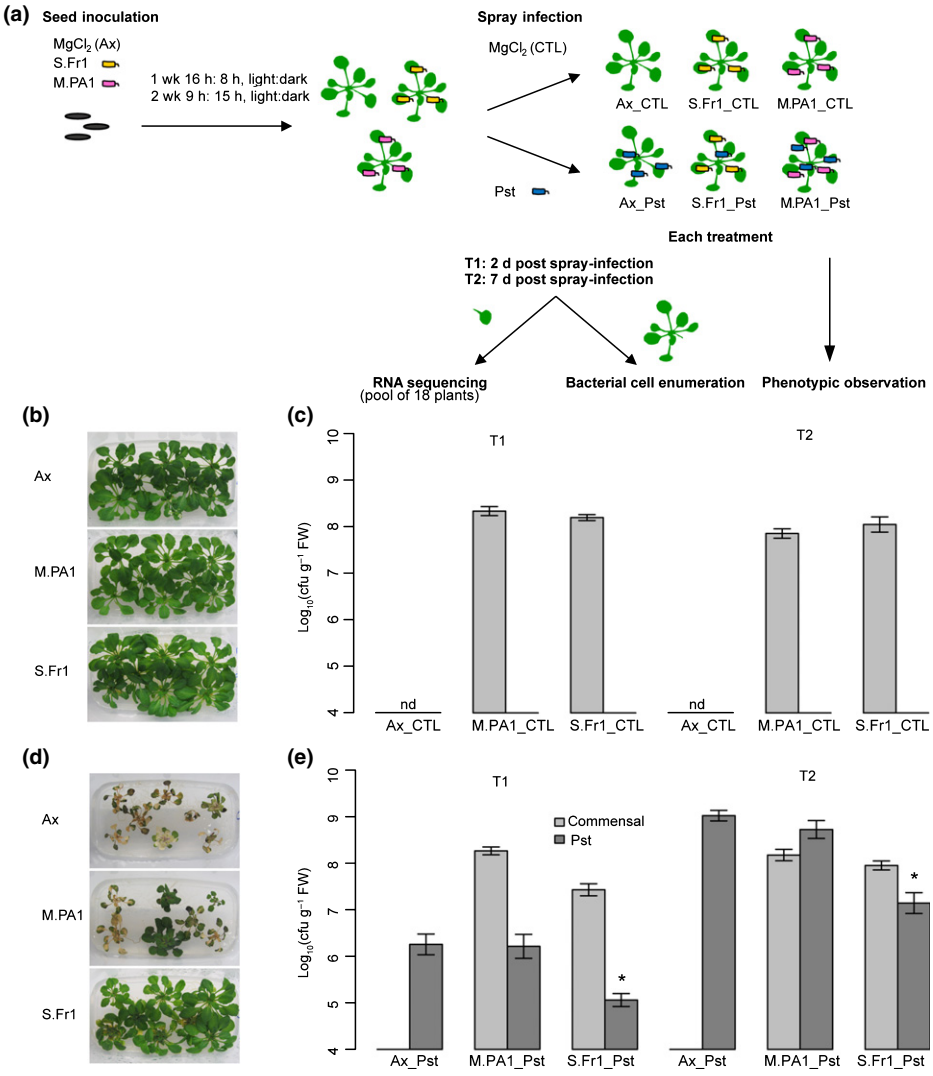

Fig. 1 *Arabidopsis thaliana* colonization by *Sphingomonas melonis* Fr1 (S.Fr1), *Methylobacterium extorquens* PA1 (M.PA1) and *Pseudomonas syringae* DC3000 (Pst). (a) Schematic of the experimental design of one biological replicate; in total, four independent experiments were conducted. Suspensions of S.Fr1, M.PA1 or 10 mM $MgCl_2$ (Ax) were applied to seeds. Twenty-one-day-old plants were spray-infected with Pst (d, e) or mock-treated with 10 mM $MgCl_2$ (b, c). Photographs were taken at 21 d post spray-infection (b, d). At T1 and T2, single leaves were sampled from plants and pooled for RNA extraction. Bacterial phyllosphere colonization of the different strains was enumerated on the remainder and is depicted as $\log_{10}$-transformed colony-forming units (CFU) per gram fresh weight (c, e). Shown are the mean ± 1SE of one representative experiment. Significantly different Pst cell numbers compared to control plants (ANOVA, *post-hoc* Tukey HSD test): *, $P < 0.05$. nd, not detected.

Consistent gene regulation in response to commensal colonization vs dynamic pathogen-induced transcriptional responses

In order to examine how consistent the changes in gene expression patterns of the treatments were between the two time points, the $\log_2$FCs for these two-group contrasts were compared against each other. Because the Arabidopsis transcriptome is expected to vary to some extent between the two time points (see also Baerenfaller *et al.*, 2012), we plotted the relative expression ratios between colonized and axenic plants (Fig. S3). Changes induced by S.Fr1 colonization showed a good correlation between the two time points with 376 genes being consistently regulated (FDR < 0.05, FC > 2 at one time point and > 1.5 at the other time point; Fig. S3a; Table S7-1). Therefore, we conclude that S.Fr1 colonization induced stable gene expression changes. In contrast to S.Fr1, M.PA1 colonization hardly influenced the Arabidopsis transcriptome. Over 30 genes were differentially expressed at T1 but only five genes were consistently regulated at both time points (Fig. S3b; Table S7-2).

For Pst-infected plants, the majority of DEGs at T1 (93%) were also regulated at T2, with many more genes additionally regulated at T2. A fraction of these (194) were also weakly induced at T1 (FC > 1.5, FDR < 0.05), indicating that the response to Pst was further augmented between T1 and T2 (Fig. S3c; Table S7-3). This shift in the gene expression profiles of Pst-infected plants can be explained by the massive proliferation of the pathogen between the two time points (Fig. 1e). Higher pathogen titers as well as higher concentrations of pathogen-associated molecular patterns (PAMPs) can induce a stronger transcriptional response (Thilmony *et al.*, 2006; Denoux *et al.*, 2008). Also, by T2 phenotypic differences are evident between Pst-infected and axenic plants (Fig. S1), suggesting that some of the regulated genes are the consequence of pathogen reprogramming.

Changes in response to M.PA1 colonization are related to oxidative stress and possibly copper homeostasis

Among the few upregulated genes in response to M.PA1 colonization were genes encoding two copper/zinc dismutases CSD1

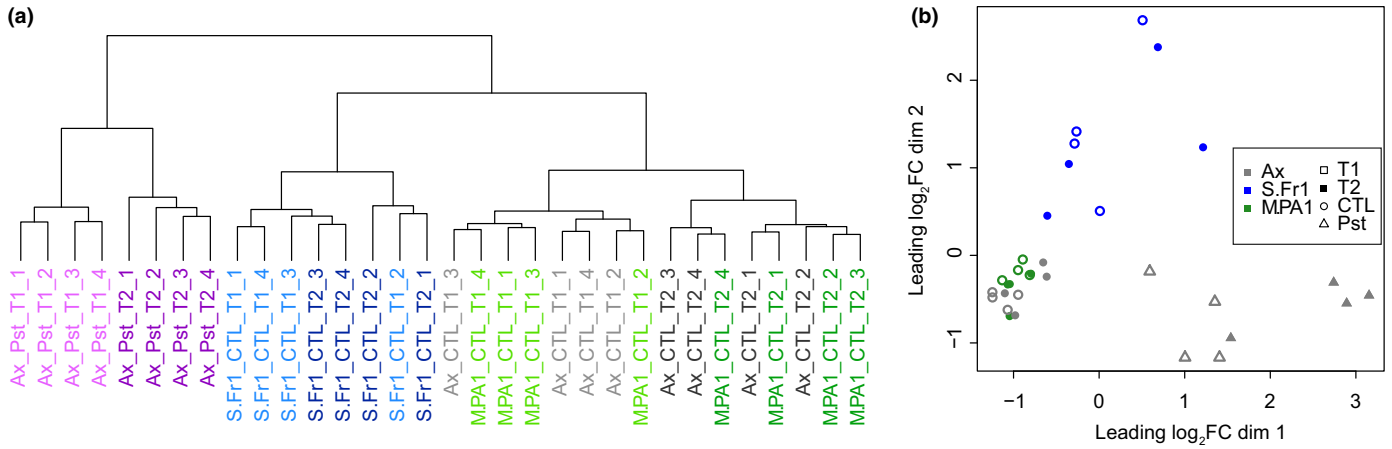

Fig. 2 Leaf transcriptomes of *Arabidopsis thaliana* colonized by *Sphingomonas melonis* Fr1 (S.Fr1) form a cluster distinct from axenic and *Methylobacterium extorquens* PA1 (M.PA1)-colonized plants and from plants infected with *Pseudomonas syringae* DC3000 (Pst). (a) Hierarchical clustering of moderated $\log_2$-transformed counts per million (cpm) for all genes with at least 0.5 cpm in at least four samples. Samples were clustered according to Ward's method based on Euclidian distances. Sample names correspond to seed-inoculation_spray-infection_time point_replicate. (b) Multidimensional scaling plot of axenic plants and plants colonized by single bacterial species. Colors reflect seed-inoculation with gray corresponding to 10 mM $MgCl_2$ (Ax), blue to S.Fr1 and green to M.PA1. Shapes depict spray-infection after 21 d with circles corresponding to mock-treatment with 10 mM $MgCl_2$ (CTL) and triangles to infection with Pst. Fillings depict the time points with empty corresponding to T1 and filled to T2.

Table 1 Number of genes differentially regulated in *Arabidopsis thaliana* leaves in response to bacterial colonization

	T1			T2		
Comparison	Total	Up	Down	Total	Up	Down
S.Fr1_CTL vs Ax_CTL	617	552	65	421	374	47
M.PA1_CTL vs Ax_CTL	37	33	4	6	2	4
Ax_Pst vs Ax_CTL	416	411	5	1809	1285	524
Comparison	Total	Up	Down	Total	Up	Down
S.Fr1_Pst vs S.Fr1_CTL	65	63	2	398	388	10
S.Fr1_Pst vs Ax_CTL	779	656	123	1193	1060	133
M.PA1_Pst vs M.PA1_CTL	332	328	4	1041	924	117
M.PA1_Pst vs Ax_CTL	422	396	26	1007	831	176

Samples were taken at T1 and T2 corresponding to 2 and 7 d post spray-infection with *Pseudomonas syringae* DC3000 (Pst) or mock-treatment (CTL). *Sphingomonas melonis* Fr1 (S.Fr1) and *Methylobacterium extorquens* PA1 (M.PA1) were seed-inoculated. Genes were considered differentially expressed in a comparison when the fold change was > 2 and the false-discovery rate was < 0.05. Ax corresponds to seed-inoculation with 10 mM $MgCl_2$.

and CSD2 and the corresponding chaperone CCS which are important for oxidative stress tolerance (Sunkar *et al.*, 2006). All three genes are targets of the microRNA miR398 (Sunkar *et al.*, 2006; Bouche, 2010) the expression of which is downregulated by biotic and abiotic stresses (Jagadeeswaran *et al.*, 2009) resulting in induction of *CSD1* and *CSD2*. Moreover, miR398 is also responsive to copper levels (Yamasaki *et al.*, 2007). Interestingly, *MIR398c* was downregulated in M.PA1-colonized plants (Table 2).

Other genes for which a downregulation in the presence of M.PA1 could be shown are responsive to copper levels as well and encode for YLS2, a transporter with homology to the maize metal-nicotianamine transporter ZmYS1 (DiDonato *et al.*, 2004; Schaaf *et al.*, 2005) and the copper transporter COPT2 (DiDonato *et al.*, 2004; del Pozo *et al.*, 2010). Together, gene expression changes in M.PA1-colonized plants are associated with oxidative stress; however, only a small number of genes was affected and these might be responsive to high copper levels. A few of these genes were also responding to S.Fr1 colonization at one time point (or in the opposite direction to Pst infection) (Table 2), which may indicate that these responses to M.PA1 colonization are not specific. Whether or not M.PA1 or other leaf bacteria trigger oxidative stress or affect copper levels in plants remains currently unknown.

S.Fr1 alters expression of genes involved in signaling and defense response

In contrast to M.PA1 colonization, many more genes were found to be responsive to S.Fr1 colonization. In total, 376 genes were consistently responding to S.Fr1 colonization with 342 up- and 34 downregulated compared to axenic controls (Table S7-1). The Arabidopsis Information Resource functional categorization tool was used to analyze S.Fr1 DEGs (Fig. 4). In the biological process category, many genes were associated with GO-terms *response to abiotic or biotic stimulus*, *response to stress*, *transport* and *signal transduction*. In the molecular function category, genes associated with the terms *kinase activity* and *transferase activity* as well as *nucleotide binding* and *other binding* were enriched. Other functional categories included cellular compartments *cell wall*, *plasma membrane*, *nucleus* and *other membranes*. For the upregulated genes, among the most significantly enriched GO-terms were plant responses to pathogen infection and intra- and intercellular communication (Table S3). Together, the DEG response

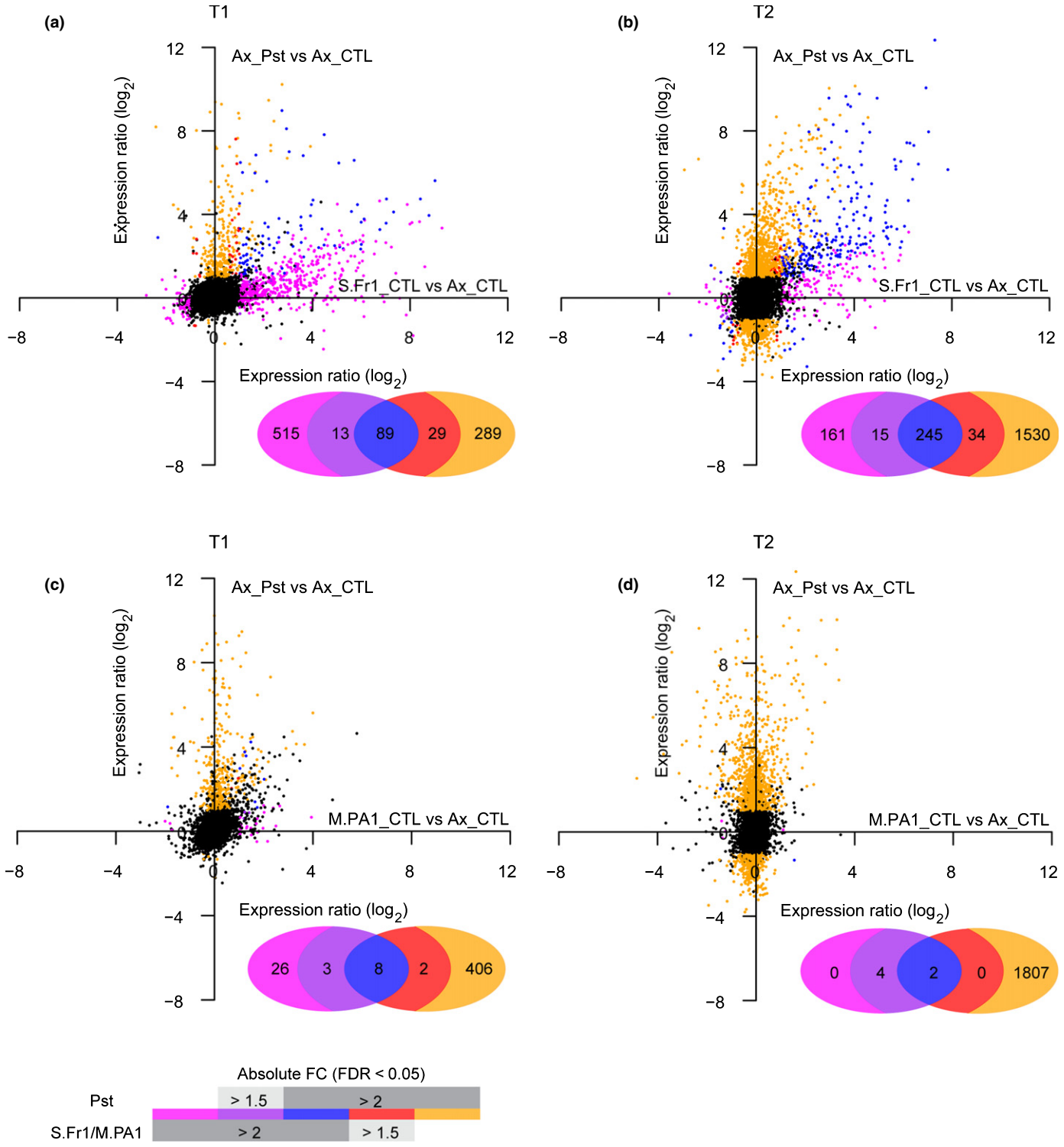

Fig. 3 Comparison of *Arabidopsis thaliana* leaf transcript profiles of commensal-colonized plants to plants infected with *Pseudomonas syringae* DC3000 (Pst). Transcript profiles of *Sphingomonas melonis* Fr1 (S.Fr1)-colonized (a, b) or *Methylobacterium extorquens* PA1 (M.PA1)-colonized (c, d) plants compared to plants infected with Pst. Expression ratios relative to control plants were calculated using EDGER and are plotted against each other at time point T1 (a, c) and T2 (b, d), corresponding to 2 and 7 d post spray-infection with Pst or mock-treatment with 10 mM $MgCl_2$ (CTL). Black dots, genes that did not show changes in gene expression; blue dots, significantly regulated in both comparisons (false discovery rate (FDR) < 0.05, absolute fold change (FC) > 2); purple dots, significantly regulated in response to commensal colonization (FDR < 0.05, absolute FC > 2) and slightly in response to Pst-infection (FDR < 0.05, absolute FC > 1.5); red dots, significantly regulated in Pst-infected plants and slightly regulated in commensal-colonized plants; magenta dots, significantly regulated in commensal-colonized plants; orange dots, significantly regulated in Pst-infected plants. The simplified Venn diagrams depict the number of genes in each category.

of Arabidopsis to S.Fr1 colonization is strikingly different from M.PA1 colonization and resembles a response to pathogenic bacteria.

S.Fr1 colonization induced many possible receptors The number of DEGs and the association of >40% of them with the GO-term *response to biotic stimulus* indicated that the presence of S.Fr1 is detected by the plant. Among the genes induced at both time points, 29 encode members of the transmembrane receptor-like kinase family (RLK) (Table S8). Mainly RLKs belonging to the family DUF26, called cysteine-rich receptor like kinases (CRK), responded to S.Fr1 colonization. The Arabidopsis genome encodes over 40 CRKs (Chen, 2001; Wrzaczek *et al.*, 2010) and 14 of these were consistently upregulated in S.Fr1-colonized plants. Several members of the CRK group are induced by reactive oxygen species, salicylic acid (SA) or pathogen infection (Chen *et al.*, 2003, 2004; Wrzaczek *et al.*, 2010). The signature tool of Genevestigator (Hruz *et al.*, 2008) was used to find experiments showing similar induction of S.Fr1-responsive CRKs. Among these were several microbe-associated molecular pattern (MAMP) treatments (e.g. elf18, flg22), plant pathogen and other treatments known to induce SA-associated responses (e.g. *G. orontii*, *P. syringae*, imidacloprid) as well as direct treatment with SA or SA-analogs and treatments inducing oxidative stress (e.g. ozone treatment, iron deficiency) (Fig. S4). Notably, effects on plant health have been described for several

Table 2 Selected genes of *Arabidopsis thaliana* responding to colonization by single bacteria discussed in the text

		S.Fr1_CTL vs Ax_CTL				M.PA1_CTL vs Ax_CTL				Ax_Pst vs Ax_CTL			
AGI	Name	log_2FC T1		log_2FC T2		log_2FC T1		log_2FC T2		log_2FC T1		log_2FC T2	
M.PA1-regulated													
AT1G08830	CSD1	0.5		**1.2**	***	**1.7**	***	**1.1**	**	0.0		0.1	
AT2G28190	CSD2	0.5		**1.3**	**	**2.2**	***	**1.5**	**	−0.5		**−1.3**	***
AT1G12520	CCS	0.5		0.9	**	**1.7**	***	0.9	*	−0.5		−0.5	*
AT5G14565	MIR398c	0.0		−0.8		**−1.8**	***	**−1.4**	*	0.4		0.5	
AT3G46900	COPT2	0.1		−0.8	*	−1.2		**−1.2**	**	0.0		0.0	
AT5G24380	YSL2	0.1		−0.6		**−1.9**	***	**−1.4**	***	**1.2**	**	**2.0**	***
RLKs													
AT4G23130	CRK5	**2.8**	***	**2.1**	***	0.5		−0.3		0.1		0.9	
AT4G23210	CRK13	**2.2**	**	**1.8**	**	−0.2		−0.4		0.8		0.3	
AT2G31880	SOBIR1	**1.3**	**	**1.4**	**	0.0		−0.2		0.3		**1.5**	***
AT2G19190	FRK1	**3.1**	***	**1.7**	**	0.1		0.5		0.7		−0.6	
AT1G51890	AT1G51890	**3.9**	***	**3.2**	***	0.6		0.1		−0.2		0.4	
Defense-related genes													
AT2G14610	PR1	**8.1**	***	**4.3**	**	2.8		−4.8		−1.8		**2.5**	*
AT3G57260	PR2	**7.0**	***	**5.7**	***	1.2		0.4		**3.6**	***	**6.2**	***
AT3G04720	PR4	**2.8**	***	**2.1**	**	0.3		0.4		1.1		0.6	
AT1G75040	PR5	**1.4**	***	**1.7**	***	0.1		−0.1		0.0		−0.5	
AT5G44420	PDF1.2	**6.1**	***	**5.2**	***	1.9		3.4		**3.8**	*	−0.1	
AT2G43570	CHI	**3.6**	***	**3.8**	***	0.7		0.9		0.4		**2.7**	***
AT1G19250	FMO1	**7.6**	***	**5.9**	**	1.6		−1.2		0.0		**6.5**	***
SA-biosynthesis and signaling													
AT1G73805	SARD1	**3.4**	***	**2.6**	***	0.0		−0.9		0.6		**2.1**	***
AT5G26920	CBP60G	**2.4**	***	**2.2**	**	−0.2		0.0		0.8		**2.0**	**
AT3G56400	WRKY70	**1.6**	***	0.9		−0.2		−0.4		0.7		**1.5**	***
AT1G74710	ICS1	0.4		0.5	*	0.0		0.1		0.1		0.4	*
AT4G39030	EDS5	**1.1**	*	**1.4**	*	−0.2		0.1		**1.7**	**	**2.4**	***
Camalexin biosynthesis													
AT4G39950	CYP79B2	0.6	**	0.3		−0.1		−0.2		**1.5**	***	**1.3**	***
AT2G22330	CYP79B3	0.3		0.0		−0.2		0.0		**1.9**	***	**1.6**	***
AT2G30750	CYP71A12	**6.0**	***	**3.5**	***	1.2		−0.7		**2.6**	*	**2.6**	**
AT2G30770	CYP71A13	**3.3**	***	1.1		−0.1		−0.9		0.6		**1.8**	**
AT3G26830	PAD3	**6.1**	***	**5.7**	***	2.4		0.1		1.1		**6.6**	***
JA-biosynthesis and signaling													
AT5G42650	AOS	−0.2		−0.1		0.0		0.1		**2.0**	***	**2.9**	***
AT3G25760	AOC1	0.3		0.2		0.1		0.3		**3.8**	***	**5.0**	***
AT3G45140	LOX2	**1.0**	***	0.7		0.1		0.0		**3.3**	***	**3.9**	***
AT1G32640	MYC2	0.2		−0.1		0.0		0.0		**2.0**	***	**2.8**	***
AT5G24780	VSP1	0.9	**	**2.1**	*	0.0		−0.8		**7.6**	***	**8.0**	***
AT2G30770	VSP2	**3.3**	***	1.1		−0.1		−0.9		0.6		**1.8**	**
AT1G72260	THI2.1	2.2		0.5		1.1		−2.1		**9.5**	***	**5.9**	***

Shown are the log_2-transformed fold changes (log_2FC) for the different comparisons at T1 and T2. Asterisks denote whether the difference in gene expression was significant (FDR < 0.001:***, < 0.01:**, < 0.05*). Absolute log_2FC > 1 and FDR < 0.05 are bold. AGI codes in bold: qPCRs were performed (see Supporting Information Table S5). For a complete list of all genes see Table S7.

S.Fr1-induced *CRK* genes (Chen *et al.*, 2003, 2004; Acharya *et al.*, 2007; Yeh *et al.*, 2015). In addition, 13 genes for LRR-type RLKs, as well as several WAK-like RLKs potentially involved in pattern recognition, were induced upon S.Fr1 colonization (Table S8).

The induction of RLK genes in response to PAMPs and pathogens has been described before (Zipfel *et al.*, 2004; Thilmony *et al.*, 2006) and some of these genes (i.e. *FRK1*, *AT1G51890*, *AT5G25930* and *AT4G18250*) also were consistently found to respond to colonization with *P. fluorescens* SS101

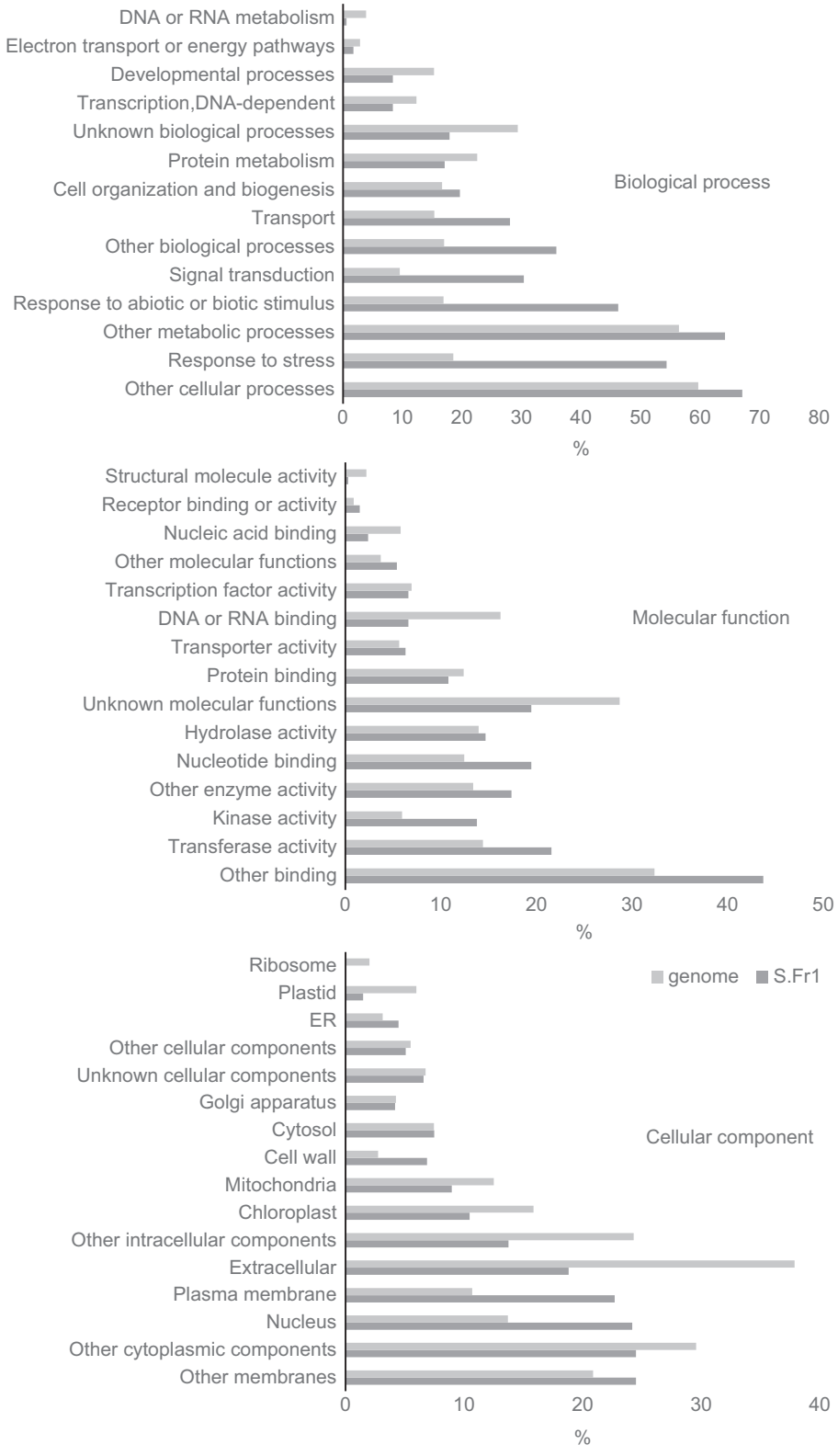

Fig. 4 Functional categorization of *Arabidopsis thaliana* genes consistently regulated by *Sphingomonas melonis* Fr1 (S.Fr1). Genes consistently differentially expressed in leaves of plants colonized by S.Fr1 compared to axenic plants (false discovery rate (FDR) < 0.05, absolute fold change (FC) > 2 at one time point and FC > 1.5 at the other time point) were subjected to functional categorization at www.arabidopsis.org. Shown are the percentages of genes annotated to the different categories in the whole genome (light gray) and in S.Fr1-regulated genes (dark gray).

(van de Mortel *et al.*, 2012). In addition to the induction of several RLK genes, *ACD6* showed increased expression as well. It was shown previously that SA affects the abundance of pattern-recognition receptors (PRRs) and the responsiveness to PAMPs (flg22 and elf18) in a positive feedback loop with ACD6 (Tateda *et al.*, 2014). The increased expression of both *ACD6* and several RLK genes encoding potentially functional PRRs could indicate a higher PRR abundance at the plasma membrane and potentially higher responsiveness to MAMP-triggering in S.Fr1-colonized plants. In addition to direct binding to RLKs and signal transduction via co-receptor binding, receptor-like proteins (RLPs) that lack the cytoplasmic kinase domain of RLKs are involved in pathogen recognition (Liebrand *et al.*, 2013; Zhang *et al.*, 2013; Fradin *et al.*, 2014; Gust & Felix, 2014). Several RLP genes as well as the gene encoding the co-receptor SOBIR1 (Liebrand *et al.*, 2014) were induced in S.Fr1-colonized plants (Tables S7-1, S8).

S.Fr1 colonization induces 'defense response genes' Several genes encoding pathogenesis-related (PR) proteins and potential antimicrobial proteins were induced by S.Fr1 colonization. Among them were marker genes for SA signaling and systemic acquired resistance (SAR) *PR1*, *PR2* and *PR5*, ethylene (ET)-responsive proteins *PR4* and *PDF1.2*, and several chitinases (Tables 2, S7-1). Three vacuolar sorting receptors (*VSR5–7*) were also among the induced genes in S.Fr1-colonized plants. *VSR6* and *VSR7* are transcriptionally induced by NPR1 (Wang *et al.*, 2005a) and were suggested to be involved in sorting of NPR1-dependent induced antimicrobial proteins upon pathogen challenge or SA accumulation (Zouhar *et al.*, 2010). Because several genes encoding PR proteins and antimicrobials were induced in S.Fr1-inoculated plants, this might suggest an increased exudation of these. Additional known SA-responsive genes were differentially regulated in S.Fr1-colonized plants as well, including genes in the significantly enriched GO-term *SA biosynthetic genes* such as the transcription factors *CBP60G*, *SARD1* and the SA-transporter *EDS5* (Zhang *et al.*, 2010; Serrano *et al.*, 2013).

Genes for other transcription factors were differentially regulated in S.Fr1-colonized plants, too, and many of these belong to families that previously have been found to be implicated in stress responses such as the WRKY, ERF, MYB and NAC transcription factor families (Table S9). Some of these showed differential expression at both time points whereas others such as *WRKY70*, which is as a positive regulator of SA-mediated signaling and a negative regulator of jasmonic acid (JA)-mediated signaling (Li *et al.*, 2004), were significantly induced at T1.

Camalexin biosynthetic genes are induced in S.Fr1-colonized plants Phytoalexins such as camalexin are low molecular weight antimicrobial peptides produced in response to several biotic and abiotic stresses (Hagemeier *et al.*, 2001; Ahuja *et al.*, 2012). Several genes implicated in camalexin biosynthesis showed higher expression in S.Fr1-colonized plants compared to axenic plants at both time points (*CYP71A12, GSTF6* and *PAD3*) and T1 only (*CYP71A13*) (Tables 2, S7-1). Consistent with our transcriptome data, camalexin was detected on S.Fr1-colonized Arabidopsis leaves in an independent study (Ryffel *et al.*, 2016), indicating that the pathway is at least locally active in leaves. The importance of camalexin in plant resistance to different pathogens varies. Although camalexin has not been found to be a determinant of resistance in the *P. syringae*–Arabidopsis interaction, it is effective in resistance to a range of other pathogens (Glazebrook & Ausubel, 1994; Zhou *et al.*, 1999; Ferrari *et al.*, 2007; Ahuja *et al.*, 2012).

Pst infection shows a strong JA response reminiscent of a COR-induced response

Next, we analyzed Arabidopsis leaves spray-infected with a low dose of the foliar pathogen Pst to mimic natural infection. Because we expected to have fewer plant cells in direct contact with the pathogen, we followed the response to the pathogen at 2 and 7 d post infection, which is in contrast to most other studies where transcriptional responses to Pst were studied after shorter pathogen exposure and usually after infiltration. Despite the variation in the output observed between the two time points, which can be explained by the different stages of infection (see above), we found 589 DEGs consistently regulated (Fig. S3c). The terms most significantly enriched for genes upregulated after Pst infection were related to immune system process and JA (Tables S2, S4). *P. syringae* produces the phytotoxin coronatine (COR), which influences several aspects of infection such as the reopening of stomata to facilitate invasion, bacterial growth and disease symptom development (Mittal & Davis, 1995; Brooks *et al.*, 2004, 2005; Melotto *et al.*, 2006) and represents a mimic of the JA conjugate JA-Ile (Zhao *et al.*, 2003; Uppalapati *et al.*, 2005; Thilmony *et al.*, 2006; Katsir *et al.*, 2008). The large fraction of JA-responsive genes induced by Pst is therefore consistent with coronatine production by the pathogen (Thilmony *et al.*, 2006). Among them are genes for the transcription factor MYC2, *VSP1/2, THI2.1* as well as JA biosynthetic genes (e.g. *LOX2-4*, *AOS*, *AOC1-4*) (Tables 2, S7-3). The increased JA signaling is thought to antagonize SA-dependent defense responses, which are effective mainly against biotrophic and hemibiotrophic pathogens (Glazebrook, 2005).

Also strongly induced by Pst infection were anthocyanin biosynthetic genes and regulators thereof (Table S7-3). Anthocyanins are stress pigments and production in response to JA or application of COR has been described (Bent *et al.*, 1992; Feys *et al.*, 1994), further hinting towards a strong coronatine response.

Transcriptional responses to S.Fr1 colonization show overlap with progressed Pst infection

Next, we compared the responses of Arabidopsis to S.Fr1 colonization and Pst infection. A heatmap of the constitutive DEGs revealed that most genes responding to S.Fr1 colonization were also responding to Pst-infected plants at T2 (Fig. 5, Tables S7, S10). The DEGs were separated into six clusters. Clusters 1 and 2 contain DEGs responding only to S.Fr1 colonization: Cluster 1 comprises 23 genes with lower expression in S.Fr1-colonized

plants as opposed to axenic, M.PA1- and Pst-colonized plants, whereas Cluster 2 (52 genes) contains genes induced specifically in response to S.Fr1 colonization. More than half of the genes in Cluster 2 belong to the GO-term *response to stimulus*, including genes for the pathogenesis-related proteins PR5, LTP2 and LTP4, a chitinase family protein and MLO12 as well as some RLKs such as FRK1 (Tables S7-1, S11-1). A third cluster contains 191 genes (Cluster 3) higher expressed in S.Fr1-colonized plants but induced at a low level by Pst at T2 compared to the other two treatments, although with some variation. This cluster contains 105 genes annotated to the GO-term category *response to stimulus* and 72 of these were annotated *defense response* (Table S11-2). Many genes for RLKs and some RLPs are in this category as well as genes related to SA biosynthesis, metabolism or signaling. In contrast to Cluster 3, genes in Cluster 4 showed higher expression in S.Fr1-colonized plants and in Pst-infected plants at T2. In this cluster (68 genes), the most significantly enriched GO-terms are *systemic acquired resistance* and *defense response, incompatible interaction* (Table S11-3). Cluster 5 genes (31) are more strongly induced in response to Pst infection than in response to S.Fr1 colonization. This cluster contains, among others, some JA-responsive genes (Table S11-4). Cluster 6 contains 11 genes downregulated in response to S.Fr1 and at T2 in response to Pst as well. In conclusion, the heatmap confirmed that some DEGs responded solely to S.Fr1 colonization, whereas the majority of DEGs were also affected in Pst-infected plants, in particular at T2.

The effect of S.Fr1 colonization on expression changes in response to Pst infection

In order to understand how commensal colonization may affect the plant response to pathogen infection, we investigated the transcriptome response of Arabidopsis to Pst infection as a function of prior colonization by S.Fr1 or M.PA1. Plant colonization by several *Sphingomonas* members has been shown to protect Arabidopsis from infection by Pst, whereas colonization with representatives of *Methylobacterium* did not (Innerebner *et al.*, 2011). As expected, seed-inoculation with S.Fr1 but not with M.PA1 reduced pathogen proliferation and disease symptoms of plants upon challenge with Pst compared to uninoculated plants (Fig. 1). Differences were also evident in the transcriptional response. Clustering of the 1000 most variable genes indicated three main clusters, one comprising axenic and M.PA1-colonized plants, one comprising axenic and M.PA1-colonized plants infected with Pst, and one comprising S.Fr1-colonized plants with and without Pst (Fig. S5a). An MDS plot encompassing all of the samples separated in the first dimension axenic and M.PA1-colonized plants not challenged by Pst from S.Fr1-inoculated and from Pst-infected plants, whereas the second dimension separated S.Fr1-inoculated plants with and without Pst challenge from all others, similar to the single bacterial treatments (Fig. S5b).

Colonization by some rhizobacteria or local infection have been found to trigger induced systemic resistance (ISR) and SAR, two forms of systemic resistance that depend on JA/ET and on SA, respectively (Fu & Dong, 2013; Pieterse *et al.*, 2014). Whereas almost no transcriptional reprogramming is observed in systemic tissue in unchallenged ISR plants, SAR-induced plants show transcriptional induction of different defense genes such as the marker genes *PR1*, *PR2*, *PR5* or *FMO1* before secondary pathogen challenge (Fu & Dong, 2013). Both ISR and SAR lead to stronger and/or faster induction of defense responses and enhanced pathogen resistance. We therefore tested whether the presence of S.Fr1 amplifies induction of defense responses after Pst challenge. We first identified genes induced by Pst infection in S.Fr1-colonized plants. As observed in axenic plants challenged with Pst, the response to Pst was also increased in S.Fr1-colonized plants at T2 compared to T1, although the overall response to Pst was lower than in plants that were axenic before Pst infection (control plants; Tables 1, S12; Fig. S6). We then scored genes that showed significantly different induction after Pst infection in S.Fr1-colonized plants compared to the induction in control plants (Table S12). At T2, 12 genes showed a stronger response to Pst compared to control plants and these were induced by Pst in S.Fr1-colonized plants only (Table S13). Several of these genes are known to be induced by pathogen treatment and represent interesting candidate genes that might contribute to plant protection. Overall, however, most genes responding significantly different to Pst showed no induction in S.Fr1-colonized plants after pathogen challenge by T2, which is probably due to the lower pathogen titer in S.Fr1-colonized plants and reduced infection (Fig. 1d,e). It is also noteworthy that a portion of the genes induced by Pst at T2 is already induced in the S.Fr1 background and thus might not undergo additional induction.

bak1/bkk1 mutant plants are affected in plant protection by S.Fr1

As described above, plants apparently detect the presence of S.Fr1 and increased expression of defense-related genes might be involved in plant protection by S.Fr1. To test for impaired S.Fr1-mediated plant protection we chose plant mutants affected in known defense signaling pathways. We analyzed whether plant protection by S.Fr1 depends on SA or JA signaling because these plant hormones are required for plant-mediated resistance in other beneficial plant–microbe interactions (e.g. Pieterse *et al.*, 1998; van de Mortel *et al.*, 2012). Neither the SA biosynthesis (*sid2*) nor the SA signaling (*eds1/pad4*, *npr1*) or JA-signaling (*jar1*) mutants tested showed defects in plant protection by S.Fr1 (Figs 6, S7a), indicating that either a combination of several hormone pathways or different signaling mechanisms are involved in the plant-mediated response. It is noteworthy that induced resistance to different pathogens upon elicitor treatment is also not affected in SA- and JA-signaling mutants (Zipfel *et al.*, 2004; Ferrari *et al.*, 2007).

We also tested FLS2 as the best studied PRR that recognizes flagellin as a MAMP/PAMP (Gomez-Gomez & Boller, 2000) but did not observe impaired plant protection by S.Fr1 (data not shown). This result might not be surprising, however, because we could not detect flagellin from *Sphingomonas* spp. in proteomics

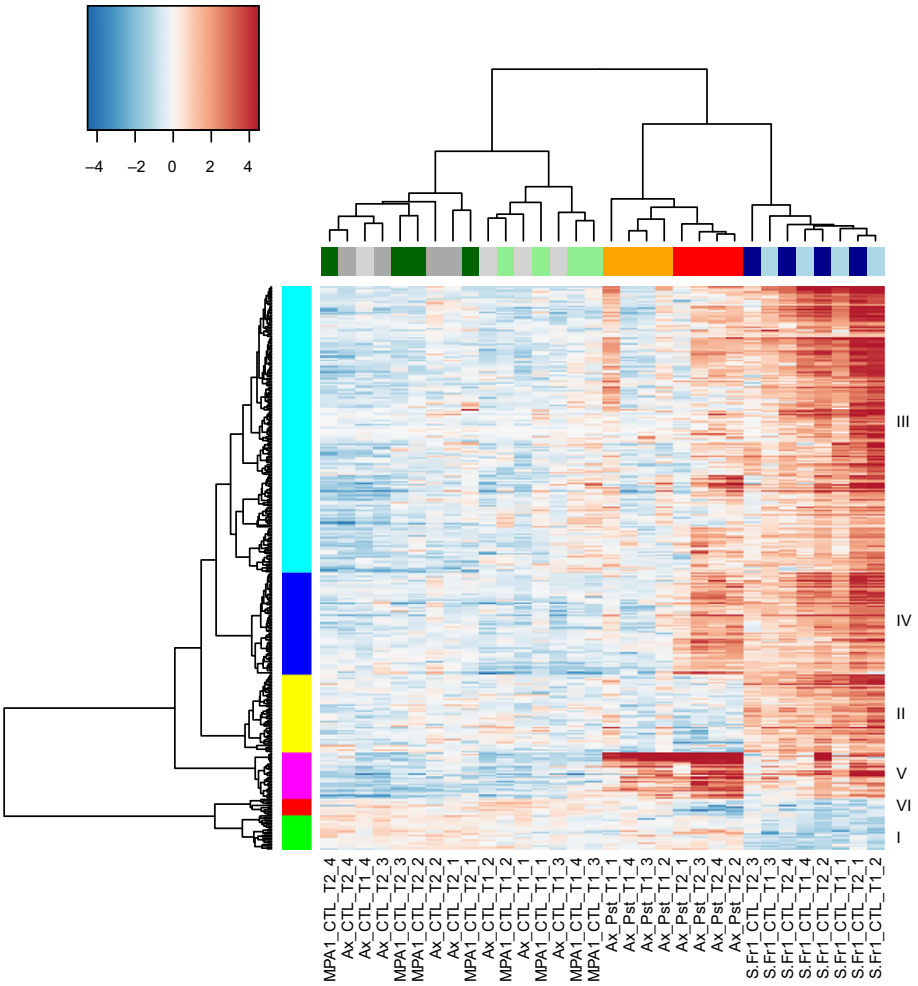

Fig. 5 Heatmap of moderated log_2-transformed counts per million for genes consistently regulated by *Sphingomonas melonis* Fr1 (S.Fr1) colonization (false discovery rate (FDR) < 0.05, absolute fold change (FC) compared to axenic controls > 2 at one time point and > 1.5 at the other time point) in *Arabidopsis thaliana* leaves. The rows were scaled by subtracting the row median. The samples were clustered according to Ward's method using as a distance measure 1 minus Pearson's correlation. The colors of the columns represent the different treatments. Plants were seed-inoculated with S.Fr1, *Methylobacterium extorquens* PA1 (M.PA1) or 10 mM $MgCl_2$ (Ax) and sprayed after 3 wk with a suspension of *Pseudomonas syringae* DC3000 (Pst) or 10 mM $MgCl_2$ (CTL). Names of the samples correspond to seed-inoculation_spray-infection_time point_replicate. The row colors represent the six clusters obtained. The cluster numbers are given on the right. The color scale was fixed to −4.5 to 4.5.

studies *in planta* in contrast to flagellin from *Pseudomonas* spp. (Delmotte *et al.*, 2009; D. B. Müller and J. A. Vorholt, unpublished). Next, we tested a triple PRR mutant (Gimenez-Ibanez *et al.*, 2009) that in addition to FLS2 is impaired in EFR recognizing the bacterial elongation factor Tu (Zipfel *et al.*, 2006) and in CERK1 perceiving chitin and involved in responses to peptidoglycan (Miya *et al.*, 2007; Willmann *et al.*, 2011). We found no impaired plant protection by S.Fr1 in the *fls2/efr/cerk1* triple mutant (Fig. 6), suggesting that none of these PRRs is essential for the plant protection phenotype. We also tested T-DNA insertion mutant lines of *RLP23*, one of the strongest induced genes in S.Fr1-colonized plants (Table S7-1). An elicitor fragment found in Nep1-like proteins has recently been shown to bind to this RLP and mediate defense activation *in vivo* (Albert *et al.*, 2015). Plant protection was not affected in these mutants either (Fig. S7b,c).

SOBIR1 and BAK1 have been identified as co-receptors of several RLPs or RLPs and RLKs, respectively, and are required for induction of PTI signaling upon recognition of the cognate ligands (Roux *et al.*, 2011; Schwessinger *et al.*, 2011; Liebrand *et al.*, 2013, 2014; Zhang *et al.*, 2013, 2014; Albert *et al.*, 2015). We therefore also used the PRR co-receptor mutants *sobir1* and *bak1/bak1-like-1* (*bkk1*) to test whether plant protection by S.Fr1 requires functional PRR signaling. Plant protection by S.Fr1 was comparable in *sobir1* and wild-type (WT) plants (Fig. 6). In *bak1/bkk1* plants colonized by S.Fr1, pathogen colonization levels and disease symptoms upon pathogen infection were also reduced compared to uninoculated control plants (Figs 6, S7). However, pathogen titers in S.Fr1-colonized plants were higher in *bak1/bkk1* plants compared to WT plants and *bak1/bkk1* plants developed disease symptoms (Figs 6, S7), indicating attenuated plant protection by S.Fr1 in this mutant.

Discussion

Plants in nature are not axenic but instead are colonized by a vast array of microorganisms, raising the question of how plants respond to these commensals and distinguish between pathogenic and beneficial microorganisms. In this study, we investigated responses of Arabidopsis leaves to colonization by bacterial phyllosphere commensals, focusing on representatives of two commonly and abundantly found genera in the phyllosphere. The gene expression changes in response to the commensal bacteria showed strong and unexpected differences. Plant colonization by *Methylobacterium extorquens* PA1 (M.PA1) did not cause significant transcriptional reprogramming, whereas colonization by

Sphingomonas melonis Fr1 (S.Fr1) resulted in differential expression of several hundreds of genes. Our data indicate that Arabidopsis perceives S.Fr1 colonization and triggers gene expression changes similar to those observed during an immune response to a pathogen encounter. It remains unclear how Arabidopsis detects S.Fr1. It is possible that the differences in the response to M.PA1 and S.Fr1 might be due to different microbe-associated molecular patterns (MAMPs) or their accessibility to pattern-recognition receptors (PRRs) as infiltration with some MAMPs evokes a stronger transcriptional response than spray application (Denoux *et al.*, 2008). Both *S. melonis* and *M. extorquens* share a mainly epiphytic lifestyle although apoplastic colonization was described for both of them also, albeit at lower levels (Sy *et al.*, 2005; Innerebner *et al.*, 2011). Therefore, it is unlikely that bacterial localization and hence different access to PRRs in leaves *per se* would be the driving force for the difference in transcriptional responses between M.PA1- and S.Fr1-colonized plants. It is also not known whether S.Fr1 detection is limited to leaves or extends to roots. Under our experimental conditions, root colonization could be observed for both commensal strains. It has been shown that some rhizosphere bacteria can induce transcriptional changes in distal parts of the plant and thereby protect plants from infection (van de Mortel *et al.*, 2012; Pieterse *et al.*, 2014).

Strong induction of pattern-triggered immunity (PTI) as well as effector recognition may lead to long-lasting induction of defense genes (Mishina & Zeier, 2007; Tsuda & Katagiri, 2010). Genes for a type III secretion system, a well-described delivery system for effectors, are absent in the genome of S.Fr1, which is in contrast to *Pseudomonas fluorescens* SS101 that also caused transcriptional reprogramming after 10–18 d of plant colonization (van de Mortel *et al.*, 2012). Genes for type IV secretion systems, which is another effector delivery system (Wallden *et al.*, 2010), are present in S.Fr1. However, how far such a transport system contributes to S.Fr1 detection remains unclear.

The nature of plant protection by S.Fr1 is not known so far, but it has been suggested that several mechanisms might act in concert (Innerebner *et al.*, 2011; Vogel *et al.*, 2012). Possible mechanisms include microbial interactions (e.g. via competition for nutrients or antibiosis), or induction of a plant-mediated response helping the plant to defend itself. Our finding that the *bak1/bkk1* mutant results in a stronger pathogen infection in the presence of S.Fr1 (Figs 6, S7) supports the importance of the plant immune system, in general, and PTI signaling, in particular, for full protection by S.Fr1. Future work will focus on understanding the detection of S.Fr1 by plants, identifying plant receptors as well as their ligands and the signaling pathways

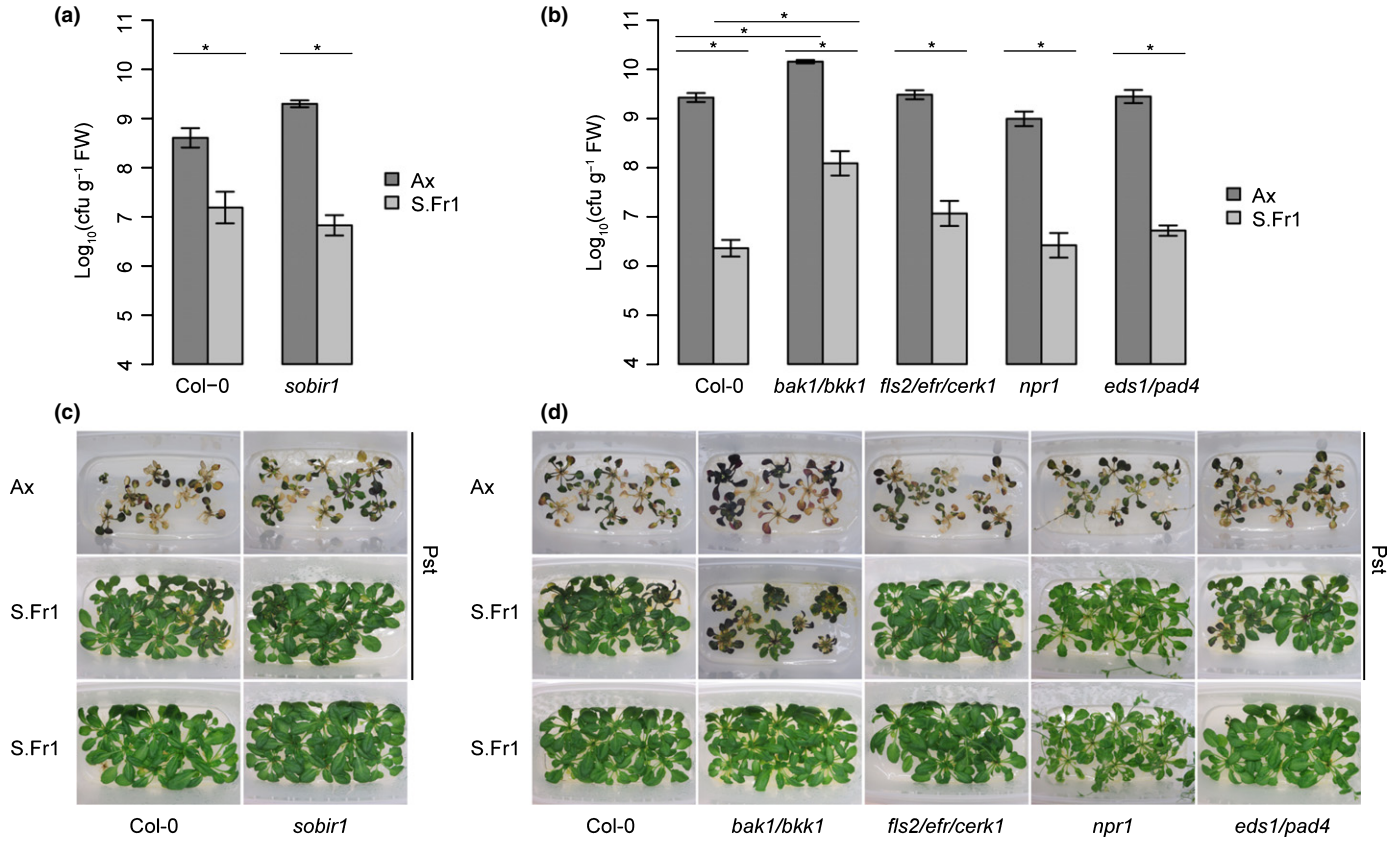

Fig. 6 *Arabidopsis thaliana bak1/bkk1* mutants show an altered plant protection phenotype by *Sphingomonas melonis* Fr1 (S.Fr1). Suspensions of S.Fr1 or 10 mM $MgCl_2$ (Ax) were applied to Col-0, *sobir1*, *bak1/bkk1*, *fls2/efr/cerk1*, *eds1/pad4* or *npr1* seeds. Twenty-one-day-old plants were spray-infected with *Pseudomonas syringae* DC3000 (Pst) or mock-treated with 10 mM $MgCl_2$. Bacterial phyllosphere colonization of the pathogen depicted as $\log_{10}$-transformed colony-forming units (CFU) per gram fresh weight at 6 (a) or 7 (b) d post infection. Shown are the mean ± 1SE of 8–9 (a) or 12 plants (b). Significantly different Pst cell numbers between comparisons (ANOVA, *post-hoc* Tukey HSD test): *, $P < 0.05$. Photographs were taken at 17 (d) or 18 (c) d post spray-infection.

employed. The finding of increased expression for several receptor-like kinases (RLKs) and receptor-like proteins (RLPs) in S.Fr1-colonized leaves provides an interesting starting point for the identification of PRRs involved in S.Fr1 detection and possibly plant protection. On the ligand side it is worth mentioning that several S.Fr1 mutants with impaired but not complete loss of plant protection against *Pseudomonas syringae* DC3000 lux (Pst) have been identified (Vogel *et al.*, 2012). Some of the targets might indeed represent promising candidates for potential MAMPs recognized directly by the plant or for enzymes, whose activity could trigger plant responses. Whether and in how far the attenuated plant protection phenotype in these S.Fr1 mutants is indeed caused by altered recognition on the plant side remains to be tested. Interestingly, *Sphingomonas* differ from other Gram-negative bacteria in that they contain glycosphingolipids in their cell envelopes (Yabuuchi *et al.*, 1990; Takeuchi *et al.*, 2001), which may also represent surface exposed potential targets for plant recognition.

Our study clearly shows that colonization by commensal bacteria can have a profound effect on the plant transcriptome. As for most transcriptome studies of Arabidopsis to biotic or abiotic stress, one has to keep in mind that a true 'untreated' control plant is difficult to obtain as a consequence of commensal colonization. Because the presence of commensals such as S.Fr1 or *P. fluorescens* SS101 potentially affects the expression of hundreds of plant genes, the phyllosphere and rhizosphere microbiota in any transcriptome study could strongly influence the plant transcriptome in plants denominated as untreated control.

In conclusion, colonization by different commensal bacteria showed distinct effects on the plant transcriptional response. Whereas colonization by M.PA1 seemed to be 'invisible' to the plant in the long term and may have evolved a strategy of MAMP recognition evasion, S.Fr1 triggered substantial plant responses. It will be interesting to identify the drivers of these differing responses and to determine whether plant responses to other common commensals are similar to the responses to M.PA1 colonization or to S.Fr1 colonization. Moreover, such studies might further increase the notion that many of the previously described pathogen-related gene responses are triggered more broadly by bacteria, which may be commensal or pathogens and are thus interesting also from an evolutionary perspective.

Acknowledgements

We thank Miriam Bortfeld-Miller for technical support. RNA sequencing was performed at the Functional Genomics Center Zurich, with support from Catharine Aquino and Lennart Opitz. Validation of RNA-Seq data was performed at the Genetic Diversity Centre, Zurich. We would like to thank Cyril Zipfel, Philippe Reymond, Thorsten Nürnberger and Christiane Nawrath for seeds and Melanie Senning for help with seed reproduction. The work was supported by a Zurich-Basel Plant Science Center – Syngenta Fellowship (to W.G. and J.A.V.), by an European Research Council Advanced Grant (PhyMo) (to J.A.V.) and by ETH Zurich.

Author contributions

C.V., N.B., W.G. and J.A.V. planned and designed the research. C.V. and N.B. performed the experiments and C.V. analyzed the data. C.V. and J.A.V. wrote the manuscript with contributions from N.B. and W.G.

References

Acharya BR, Raina S, Maqbool SB, Jagadeeswaran G, Mosher SL, Appel HM, Schultz JC, Klessig DF, Raina R. 2007. Overexpression of CRK13, an Arabidopsis cysteine-rich receptor-like kinase, results in enhanced resistance to *Pseudomonas syringae*. *Plant Journal* **50**: 488–499.

Ahuja I, Kissen R, Bones AM. 2012. Phytoalexins in defense against pathogens. *Trends in Plant Science* **17**: 73–90.

Albert I, Böhm H, Albert M, Feiler CE, Imkampe J, Wallmeroth N, Brancato C, Raaymakers TM, Oome S, Zhang H *et al.* 2015. An RLP23–SOBIR1–BAK1 complex mediates NLP-triggered immunity. *Nature Plants* **1**: 15140.

Baerenfaller K, Massonnet C, Walsh S, Baginsky S, Buhlmann P, Hennig L, Hirsch-Hoffmann M, Howell KA, Kahlau S, Radziejwoski A *et al.* 2012. Systems-based analysis of *Arabidopsis* leaf growth reveals adaptation to water deficit. *Molecular Systems Biology* **8**: 606.

Bent AF, Innes RW, Ecker JR, Staskawicz BJ. 1992. Disease development in ethylene-insensitive *Arabidopsis thaliana* infected with virulent and avirulent *Pseudomonas* and *Xanthomonas* pathogens. *Molecular Plant–Microbe Interactions* **5**: 372–378.

Bodenhausen N, Horton MW, Bergelson J. 2013. Bacterial communities associated with the leaves and the roots of *Arabidopsis thaliana*. *PLoS ONE* **8**: e56329.

Böhm H, Albert I, Fan L, Reinhard A, Nürnberger T. 2014. Immune receptor complexes at the plant cell surface. *Current Opinion in Plant Biology* **20**: 47–54.

Boller T, Felix G. 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annual Review of Plant Biology* **60**: 379–406.

Bouche N. 2010. New insights into miR398 functions in *Arabidopsis*. *Plant Signaling & Behavior* **5**: 684–686.

Brooks DM, Bender CL, Kunkel BN. 2005. The *Pseudomonas syringae* phytotoxin coronatine promotes virulence by overcoming salicylic acid-dependent defences in *Arabidopsis thaliana*. *Molecular Plant Pathology* **6**: 629–639.

Brooks DM, Hernandez-Guzman G, Kloek AP, Alarcon-Chaidez F, Sreedharan A, Rangaswamy V, Penaloza-Vazquez A, Bender CL, Kunkel BN. 2004. Identification and characterization of a well-defined series of coronatine biosynthetic mutants of *Pseudomonas syringae* pv. *tomato* DC3000. *Molecular Plant-Microbe Interactions* **17**: 162–174.

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P. 2013. Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology* **64**: 807–838.

Cartieaux F, Contesto C, Gallou A, Desbrosses G, Kopka J, Taconnat L, Renou JP, Touraine B. 2008. Simultaneous interaction of *Arabidopsis thaliana* with *Bradyrhizobium* sp. strain ORS278 and *Pseudomonas syringae* pv. *tomato* DC3000 leads to complex transcriptome changes. *Molecular Plant–Microbe Interactions* **21**: 244–259.

Cartieaux F, Thibaud MC, Zimmerli L, Lessard P, Sarrobert C, David P, Gerbaud A, Robaglia C, Somerville S, Nussaume L. 2003. Transcriptome analysis of *Arabidopsis* colonized by a plant-growth promoting rhizobacterium reveals a general effect on disease resistance. *Plant Journal* **36**: 177–188.

Chen K, Du L, Chen Z. 2003. Sensitization of defense responses and activation of programmed cell death by a pathogen-induced receptor-like protein kinase in *Arabidopsis*. *Plant Molecular Biology* **53**: 61–74.

Chen K, Fan B, Du L, Chen Z. 2004. Activation of hypersensitive cell death by pathogen-induced receptor-like protein kinases from *Arabidopsis*. *Plant Molecular Biology* **56**: 271–283.

Chen Z. 2001. A superfamily of proteins with novel cysteine-rich repeats. *Plant Physiology* **126**: 473–476.

Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B, Schlapbach R, von Mering C, Vorholt JA. 2009. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proceedings of the National Academy of Sciences, USA* **106**: 16428–16433.

Denoux C, Galletti R, Mammarella N, Gopalan S, Werck D, De Lorenzo G, Ferrari S, Ausubel FM, Dewdney J. 2008. Activation of defense response pathways by OGs and Flg22 elicitors in *Arabidopsis* seedlings. *Molecular Plant* **1**: 423–445.

DiDonato RJ, Roberts LA, Sanderson T, Eisley RB, Walker EL. 2004. *Arabidopsis Yellow Stripe-Like2* (*YSL2*): a metal-regulated gene encoding a plasma membrane transporter of nicotianamine-metal complexes. *Plant Journal* **39**: 403–414.

Dodds PN, Rathjen JP. 2010. Plant immunity: towards an integrated view of plant–pathogen interactions. *Nature Reviews Genetics* **11**: 539–548.

Fan J, Crooks C, Lamb C. 2008. High-throughput quantitative luminescence assay of the growth in planta of *Pseudomonas syringae* chromosomally tagged with *Photorhabdus luminescens luxCDABE. Plant Journal* **53**: 393–399.

Ferrari S, Galletti R, Denoux C, De Lorenzo G, Ausubel FM, Dewdney J. 2007. Resistance to *Botrytis cinerea* induced in *Arabidopsis* by elicitors is independent of salicylic acid, ethylene, or jasmonate signaling but requires *PHYTOALEXIN DEFICIENT3. Plant Physiology* **144**: 367–379.

Feys BJF, Benedetti CE, Penfold CN, Turner JG. 1994. *Arabidopsis* mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. *Plant Cell* **6**: 751–759.

Fradin EF, Zhang Z, Rovenich H, Song Y, Liebrand TWH, Masini L, van den Berg GCM, Joosten MHAJ, Thomma BPHJ. 2014. Functional analysis of the tomato immune receptor Ve1 through domain swaps with Its non-functional homolog Ve2. *PLoS ONE* **9**: e88208.

Fu ZQ, Dong XN. 2013. Systemic acquired resistance: turning local infection into global defense. *Annual Review of Plant Biology* **64**: 839–863.

Gimenez-Ibanez S, Ntoukakis V, Rathjen JP. 2009. The LysM receptor kinase CERK1 mediates bacterial perception in Arabidopsis. *Plant Signaling & Behavior* **4**: 539–541.

Glazebrook J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology* **43**: 205–227.

Glazebrook J, Ausubel FM. 1994. Isolation of phytoalexin-deficient mutants of *Arabidopsis thaliana* and characterization of their interactions with bacterial pathogens. *Proceedings of the National Academy of Sciences, USA* **91**: 8955–8959.

Gomez-Gomez L, Boller T. 2000. FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis. Molecular Cell* **5**: 1003–1011.

Gust AA, Felix G. 2014. Receptor like proteins associate with SOBIR1-type of adaptors to form bimolecular receptor kinases. *Current Opinion in Plant Biology* **21**: 104–111.

Hagemeier J, Schneider B, Oldham NJ, Hahlbrock K. 2001. Accumulation of soluble and wall-bound indolic metabolites in *Arabidopsis thaliana* leaves infected with virulent or avirulent *Pseudomonas syringae* pathovar tomato strains. *Proceedings of the National Academy of Sciences, USA* **98**: 753–758.

Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, Widmayer P, Gruissem W, Zimmermann P. 2008. Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. *Advances in Bioinformatics* **2008**: 420747.

Innerebner G, Knief C, Vorholt JA. 2011. Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by *Sphingomonas* strains in a controlled model system. *Applied and Environmental Microbiology* 77: 3202–3210.

Jagadeeswaran G, Saini A, Sunkar R. 2009. Biotic and abiotic stress down-regulate miR398 expression in *Arabidopsis. Planta* **229**: 1009–1014.

Jones JDG, Dangl JL. 2006. The plant immune system. *Nature* **444**: 323–329.

Katsir L, Schilmiller AL, Staswick PE, He SY, Howe GA. 2008. COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. *Proceedings of the National Academy of Sciences, USA* **105**: 7100–7105.

Kim H, Nishiyama W, Kunito T, Senoo K, Kawahara K, Murakami K, Oyaizu H. 1998. High population of *Sphingomonas* species on plant surface. *Journal of Applied Microbiology* **85**: 731–736.

King EO, Ward MK, Raney DE. 1954. 2 simple media for the demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Clinical Medicine* **44**: 301–307.

Knief C, Frances L, Vorholt JA. 2010a. Competitiveness of diverse *Methylobacterium* strains in the phyllosphere of *Arabidopsis thaliana* and identification of representative models, including *M. extorquens* PA1. *Microbial Ecology* **60**: 440–452.

Knief C, Ramette A, Frances L, Alonso-Blanco C, Vorholt JA. 2010b. Site and plant species are important determinants of the *Methylobacterium* community composition in the plant phyllosphere. *ISME Journal* **4**: 719–728.

Leveau J. 2015. Life of microbes on aerial plant parts. In: Lugtenberg B, ed. *Principles of plant–microbe interactions.* Cham, Switzerland: Springer International Publishing, 17–24.

Li J, Brader G, Palva ET. 2004. The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. *Plant Cell* **16**: 319–331.

Liebrand TWH, van den Burg HA, Joosten MHAJ. 2014. Two for all: receptor-associated kinases SOBIR1 and BAK1. *Trends in Plant Science* **19**: 123–132.

Liebrand TWH, van den Berg GCM, Zhang Z, Smit P, Cordewener JHG, America AHP, Sklenar J, Jones AME, Tameling WIL, Robatzek S *et al.* 2013. Receptor-like kinase SOBIR1/EVR interacts with receptor-like proteins in plant immunity against fungal infection. *Proceedings of the National Academy of Sciences, USA* **110**: 10010–10015.

Lindow SE, Brandl MT. 2003. Microbiology of the phyllosphere. *Applied and Environmental Microbiology* **69**: 1875–1883.

Maere S, Heymans K, Kuiper M. 2005. BiNGO: a cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. *Bioinformatics* **21**: 3448–3449.

McCarthy DJ, Chen YS, Smyth GK. 2012. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Research* **40**: 4288–4297.

Melotto M, Underwood W, Koczan J, Nomura K, He SY. 2006. Plant stomata function in innate immunity against bacterial invasion. *Cell* **126**: 969–980.

Mishina TE, Zeier J. 2007. Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in *Arabidopsis. Plant Journal* **50**: 500–513.

Mittal S, Davis KR. 1995. Role of the phytotoxin coronatine in the infection of *Arabidopsis thaliana* by *Pseudomonas syringae* pv. *tomato. Molecular Plant-Microbe Interactions* **8**: 165–171.

Miya A, Albert P, Shinya T, Desaki Y, Ichimura K, Shirasu K, Narusaka Y, Kawakami N, Kaku H, Shibuya N. 2007. CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. *Proceedings of the National Academy of Sciences, USA* **104**: 19 613–19 618.

van de Mortel JE, de Vos RCH, Dekkers E, Pineda A, Guillod L, Bouwmeester K, van Loon JJA, Dicke M, Raaijmakers JM. 2012. Metabolic and transcriptomic changes induced in *Arabidopsis* by the rhizobacterium *Pseudomonas fluorescens* SS101. *Plant Physiology* **160**: 2173–2188.

Penuelas J, Terradas J. 2014. The foliar microbiome. *Trends in Plant Science* **19**: 278–280.

Pieterse CM, Zamioudis C, Berendsen RL, Weller DM, Van Wees SC, Bakker PA. 2014. Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology* **52**: 347–375.

Pieterse CMJ, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SCM. 2012. Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology* **28**: 489–521.

Pieterse CMJ, van Wees SCM, van Pelt JA, Knoester M, Laan R, Gerrits N, Weisbeek PJ, van Loon LC. 1998. A novel signaling pathway controlling induced systemic resistance in Arabidopsis. *Plant Cell* **10**: 1571–1580.

del Pozo T, Cambiazo V, Gonzalez M. 2010. Gene expression profiling analysis of copper homeostasis in *Arabidopsis thaliana. Biochemical and Biophysical Research Communications* **393**: 248–252.

Ritpitakphong U, Falquet L, Vimoltust A, Berger A, Metraux JP, L'Haridon F. 2016. The microbiome of the leaf surface of Arabidopsis protects against a fungal pathogen. *New Phytologist* **210**: 1033–1043.

Robinson MD, McCarthy DJ, Smyth GK. 2010. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**: 139–140.

Roux M, Schwessinger B, Albrecht C, Chinchilla D, Jones A, Holton N, Malinovsky FG, Tor M, de Vries S, Zipfel C. 2011. The Arabidopsis leucine-rich repeat receptor-like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. *Plant Cell* **23**: 2440–2455.

Ryffel F, Helfrich EJ, Kiefer P, Peyriga L, Portais JC, Piel J, Vorholt JA. 2016. Metabolic footprint of epiphytic bacteria on *Arabidopsis thaliana* leaves. *ISME Journal* **10**: 632–643.

Schaaf G, Schikora A, Haberle J, Vert G, Ludewig U, Briat JF, Curie C, von Wiren N. 2005. A putative function for the *Arabidopsis* Fe-phytosiderophore transporter homolog AtYSL2 in Fe and Zn homeostasis. *Plant Cell Physiology* **46**: 762–774.

Schlaeppi K, Bulgarelli D. 2015. The plant microbiome at work. *Molecular Plant-Microbe Interactions* **28**: 212–217.

Schwessinger B, Roux M, Kadota Y, Ntoukakis V, Sklenar J, Jones A, Zipfel C. 2011. Phosphorylation-dependent differential regulation of plant growth, cell death, and innate immunity by the regulatory receptor-like kinase BAK1. *PLoS Genetics* 7: e1002046.

Serrano M, Wang BJ, Aryal B, Garcion C, Abou-Mansour E, Heck S, Geisler M, Mauch F, Nawrath C, Metraux JP. 2013. Export of salicylic acid from the chloroplast requires the multidrug and toxin extrusion-like transporter EDS5. *Plant Physiology* **162**: 1815–1821.

Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T. 2011. Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics* **27**: 431–432.

Sunkar R, Kapoor A, Zhu JK. 2006. Posttranscriptional induction of two Cu/Zn superoxide dismutase genes in *Arabidopsis* is mediated by downregulation of miR398 and important for oxidative stress tolerance. *Plant Cell* **18**: 2051–2065.

Supek F, Bosnjak M, Skunca N, Smuc T. 2011. REVIGO summarizes and visualizes long lists of gene ontology terms. *PLoS ONE* **6**: e21800.

Sy A, Timmers ACJ, Knief C, Vorholt JA. 2005. Methylotrophic metabolism is advantageous for *Methylobacterium extorquens* during colonization of *Medicago truncatula* under competitive conditions. *Applied and Environmental Microbiology* **71**: 7245–7252.

Takeuchi M, Hamana K, Hiraishi A. 2001. Proposal of the genus *Sphingomonas sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses. *International Journal of Systematic and Evolutionary Microbiology* **51**: 1405–1417.

Tao Y, Xie ZY, Chen WQ, Glazebrook J, Chang HS, Han B, Zhu T, Zou GZ, Katagiri F. 2003. Quantitative nature of *Arabidopsis* responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. *Plant Cell* **15**: 317–330.

Tateda C, Zhang ZQ, Shrestha J, Jelenska J, Chinchilla D, Greenberg JT. 2014. Salicylic acid regulates *Arabidopsis* microbial pattern receptor kinase levels and signaling. *Plant Cell* **26**: 4171–4187.

Thilmony R, Underwood W, He SY. 2006. Genome-wide transcriptional analysis of the *Arabidopsis thaliana* interaction with the plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000 and the human pathogen *Escherichia coli* O157:H7. *Plant Journal* **46**: 34–53.

Truman W, de Zabala MT, Grant M. 2006. Type III effectors orchestrate a complex interplay between transcriptional networks to modify basal defence responses during pathogenesis and resistance. *Plant Journal* **46**: 14–33.

Tsuda K, Katagiri F. 2010. Comparing signaling mechanisms engaged in pattern-triggered and effector-triggered immunity. *Current Opinion in Plant Biology* **13**: 459–465.

Uppalapati SR, Ayoubi P, Weng H, Palmer DA, Mitchell RE, Jones W, Bender CL. 2005. The phytotoxin coronatine and methyl jasmonate impact multiple phytohormone pathways in tomato. *Plant Journal* **42**: 201–217.

Verhagen BWM, Glazebrook J, Zhu T, Chang HS, van Loon LC, Pieterse CMJ. 2004. The transcriptome of rhizobacteria-induced systemic resistance in *Arabidopsis*. *Molecular Plant–Microbe Interactions* **17**: 895–908.

Vogel C, Innerebner G, Zingg J, Guder J, Vorholt JA. 2012. Forward genetic *in planta* screen for identification of plant-protective traits of *Sphingomonas* sp. strain Fr1 against *Pseudomonas syringae* DC3000. *Applied and Environmental Microbiology* **78**: 5529–5535.

Vorholt JA. 2012. Microbial life in the phyllosphere. *Nature Reviews Microbiology* **10**: 828–840.

Wallden K, Rivera-Calzada A, Waksman G. 2010. Type IV secretion systems: versatility and diversity in function. *Cellular Microbiology* **12**: 1203–1212.

Wang D, Weaver ND, Kesarwani M, Dong XN. 2005a. Induction of protein secretory pathway is required for systemic acquired resistance. *Science* **308**: 1036–1040.

Wang YQ, Ohara Y, Nakayashiki H, Tosa Y, Mayama S. 2005b. Microarray analysis of the gene expression profile induced by the endophytic plant growth-promoting rhizobacteria, *Pseudomonas fluorescens* FPT9601-T5 in *Arabidopsis*. *Molecular Plant–Microbe Interactions* **18**: 385–396.

Weston DJ, Pelletier DA, Morrell-Falvey JL, Tschaplinski TJ, Jawdy SS, Lu TY, Allen SM, Melton SJ, Martin MZ, Schadt CW *et al.* 2012. *Pseudomonas fluorescens* induces strain-dependent and strain-independent host plant responses in defense networks, primary metabolism, photosynthesis, and fitness. *Molecular Plant–Microbe Interactions* **25**: 765–778.

Willmann R, Lajunen HM, Erbs G, Newman MA, Kolb D, Tsuda K, Katagiri F, Fliegmann J, Bono JJ, Cullimore JV *et al.* 2011. Arabidopsis lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection. *Proceedings of the National Academy of Sciences, USA* **108**: 19 824–19 829.

Wrzaczek M, Brosche M, Salojarvi J, Kangasjarvi S, Idanheimo N, Mersmann S, Robatzek S, Karpinski S, Karpinska B, Kangasjarvi J. 2010. Transcriptional regulation of the CRK/DUF26 group of receptor-like protein kinases by ozone and plant hormones in *Arabidopsis*. *BMC Plant Biology* **10**: 95.

Yabuuchi E, Yano I, Oyaizu H, Hashimoto Y, Ezaki T, Yamamoto H. 1990. Proposals of *Sphingomonas paucimobilis* gen. nov. and comb. nov., *Sphingomonas parapaucimobilis* sp. nov., *Sphingomonas yanoikuyae* sp. nov., *Sphingomonas adhaesiva* sp. nov., *Sphingomonas capsulata* comb. nov., and 2 genospecies of the genus *Sphingomonas*. *Microbiology and Immunology* **34**: 99–119.

Yamasaki H, Abdel-Ghany SE, Cohu CM, Kobayashi Y, Shikanai T, Pilon M. 2007. Regulation of copper homeostasis by micro-RNA in *Arabidopsis*. *Journal of Biological Chemistry* **282**: 16 369–16 378.

Yeh YH, Chang YH, Huang PY, Huang JB, Zimmerli L. 2015. Enhanced Arabidopsis pattern-triggered immunity by overexpression of cysteine-rich receptor-like kinases. *Frontiers in Plant Science* **6**: 322.

Zhang LS, Kars I, Essenstam B, Liebrand TWH, Wagemakers L, Elberse J, Tagkalaki P, Tjoitang D, van den Ackerveken G, van Kan JAL. 2014. Fungal endopolygalacturonases are recognized as microbe-associated molecular patterns by the *Arabidopsis* receptor-like protein RESPONSIVENESS TO BOTRYTIS POLYGALACTURONASES1. *Plant Physiology* **164**: 352–364.

Zhang WG, Fraiture M, Kolb D, Loffelhardt B, Desaki Y, Boutrot FFG, Tor M, Zipfel C, Gust AA, Brunner F. 2013. *Arabidopsis* RECEPTOR-LIKE PROTEIN30 and receptor-like kinase SUPPRESSOR OF BIR1-1/EVERSHED mediate innate immunity to necrotrophic fungi. *Plant Cell* **25**: 4227–4241.

Zhang YX, Xu SH, Ding PT, Wang DM, Cheng YT, He J, Gao MH, Xu F, Li Y, Zhu ZH *et al.* 2010. Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. *Proceedings of the National Academy of Sciences, USA* **107**: 18 220–18 225.

Zhao YF, Thilmony R, Bender CL, Schaller A, He SY, Howe GA. 2003. Virulence systems of *Pseudomonas syringae* pv. *tomato* promote bacterial speck disease in tomato by targeting the jasmonate signaling pathway. *Plant Journal* **36**: 485–499.

Zhou N, Tootle TL, Glazebrook J. 1999. *Arabidopsis PAD3*, a gene required for camalexin biosynthesis, encodes a putative cytochrome P450 monooxygenase. *Plant Cell* **11**: 2419–2428.

Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JDG, Boller T, Felix G. 2006. Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. *Cell* **125**: 749–760.

Zipfel C, Robatzek S, Navarro L, Oakeley EJ, Jones JDG, Felix G, Boller T. 2004. Bacterial disease resistance in *Arabidopsis* through flagellin perception. *Nature* **428**: 764–767.

Zouhar J, Munoz A, Rojo E. 2010. Functional specialization within the vacuolar sorting receptor family: VSR1, VSR3 and VSR4 sort vacuolar storage cargo in seeds and vegetative tissues. *Plant Journal* **64**: 577–588.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Images of *Arabidopsis thaliana* plants colonized by *Sphingomonas melonis* Fr1, *Methylobacterium extorquens* PA1 and *Pseudomonas syringae* DC3000 at T2.

Fig. S2 Venn diagrams of differentially regulated genes between *Arabidopsis thaliana* plants colonized by *Sphingomonas melonis* Fr1, *Methylobacterium extorquens* PA1 or *Pseudomonas syringae* DC3000 and axenic control plants.

Fig. S3 Comparison of leaf transcript profiles in *Arabidopsis thaliana* plants colonized by *Sphingomonas melonis* Fr1, *Methylobacterium extorquens* PA1 or *Pseudomonas syringae* DC3000.

Fig. S4 Signature analysis of *Arabidopsis thaliana CRK*s responding to *Sphingomonas melonis* Fr1 colonization with Genevestigator.

Fig. S5 Hierarchical clustering and MDS plot of all *Arabidopsis thaliana* leaf transcriptome samples based on moderated $\log_2$-transformed counts per million.

Fig. S6 Comparison of leaf transcript profiles of *Sphingomonas melonis* Fr1-colonized or *Methylobacterium extorquens* PA1-colonized *Arabidopsis thaliana* plants to axenic plants in response to *Pseudomonas syringae* DC3000 infection.

Fig. S7 Plant protection by *Sphingomonas melonis* Fr1 in different *Arabidopsis thaliana* mutant plants.

Table S1 Quantification of mapped reads

Table S2 GO over-representation analysis for genes up- and downregulated in leaves of *Arabidopsis thaliana* by colonization with single bacteria

Table S3 GO over-representation analysis for genes consistently upregulated in leaves of *Arabidopsis thaliana* by *Sphingomonas melonis* Fr1 colonization

Table S4 Summarized GO-terms of biological processes enriched in genes regulated in leaves of *Pseudomonas syringae* DC3000-infected *Arabidopsis thaliana*

Table S5 Validation of RNA-Seq data with quantitative reverse transcription PCR

Table S6 Primers used for quantitative PCR validation of RNA-Seq results

Table S7 Genes differentially expressed at T1 or T2 in leaves of *Arabidopsis thaliana* plants colonized by single bacteria

Table S8 Receptor-like kinases and receptor like proteins differentially regulated in leaves of *Arabidopsis thaliana* by *Sphingomonas melonis* Fr1 colonization

Table S9 Transcription factor encoding genes differentially expressed in leaves of *Sphingomonas melonis* Fr1-colonized *Arabidopsis thaliana* at T1 or T2

Table S10 Results of all two group comparisons for differential expression analysis using EDGER

Table S11 GO over-representation analysis for clusters of genes consistently regulated in leaves of *Sphingomonas melonis* Fr1-colonized *Arabidopsis thaliana*

Table S12 Genes responding differently to *Pseudomonas syringae* DC3000 infection in leaves of *Sphingomonas melonis* Fr1-colonized *Arabidopsis thaliana* compared to axenic plants at T1 and T2

Table S13 Genes responding more strongly to infection with *Pseudomonas syringae* DC3000 in leaves of *Sphingomonas melonis* Fr1-colonized *Arabidopsis thaliana* compared to axenic plants at T2

Methods S1 RNA sequencing.

Methods S2 Validation of sequencing data with qRT-PCR.

Please note: Wiley Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.